

<b>Office Action Summary</b>	Application No.	Applicant(s)
	09/701,589	HERRERA ESTRELLA, LUIS RAFAEL
	Examiner Janell Taylor Cleveland	Art Unit 1655

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --  
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1) Responsive to communication(s) filed on 23 February 2001.

2a) This action is FINAL.      2b) This action is non-final.

3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4) Claim(s) 1-138 is/are pending in the application.

4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

5) Claim(s) \_\_\_\_\_ is/are allowed.

6) Claim(s) 1-138 is/are rejected.

7) Claim(s) \_\_\_\_\_ is/are objected to.

8) Claims \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

9) The specification is objected to by the Examiner.

10) The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

11) The proposed drawing correction filed on \_\_\_\_\_ is: a) approved b) disapproved.

12) The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

13) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) All b) Some \* c) None of:

1. Certified copies of the priority documents have been received.
2. Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

14) Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

**Attachment(s)**

15) Notice of References Cited (PTO-892)      18) Interview Summary (PTO-413) Paper No(s). \_\_\_\_\_.

16) Notice of Draftsperson's Patent Drawing Review (PTO-948)      19) Notice of Informal Patent Application (PTO-152)

17) Information Disclosure Statement(s) (PTO-1449) Paper No(s) \_\_\_\_\_.      20) Other: \_\_\_\_\_

## DETAILED ACTION

### *Claim Objections*

1. The numbering of claims is not in accordance with 37 CFR 1.126 which requires the original numbering of the claims to be preserved throughout the prosecution. When claims are canceled, the remaining claims must not be renumbered. **When new claims are presented, they must be numbered consecutively beginning with the number next following the highest numbered claims previously presented** (whether entered or not). There are no claims numbered as 103-110. Misnumbered claims 111-146 have been renumbered 103-138. All subsequent references to claim numbers refer to the newly numbered claims.
2. Claims 1-73 are objected to because of the following informalities: the word "transcription" is misspelled in line 7 of claim 1. Appropriate correction is required.
3. Claim 81 is objected to because of the following informalities: the word "dehyrdogenase" is misspelled. Appropriate correction is required.
4. Claims 129 and 132 are identical in scope. Applicant is required to cancel the claim(s), or amend the claim(s) or rewrite the claim(s) so they are no longer identical.

### *Claim Rejections - 35 USC § 112*

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.
6. The terms "better" and "higher" in claim 107 are relative terms which renders the claim indefinite. The term "better" or "higher" is not defined by the claim, the

specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Appropriate correction is required.

7. The terms "increased" and less" in claims 93-106 are relative terms which renders the claim indefinite. The terms are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Appropriate correction is required.

8. Claim 92 recites the limitation "the vector" in line 1. There is insufficient antecedent basis for this limitation in the claim because there is no mention of the word "vector" in parent claim 74.

#### ***Claim Rejections - 35 USC § 102***

9. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

10. Claims 1, 2, 5, 6, 8, 11-13, 16, 17, 19, 22, 23, 26, 27, 29, 62, 63, 66, 67, 69, 74, 75, 78, 79, 80, 82, 84, 88, 90-94, 97-99, 101, 103, 104, 107-109, 114-117, 122, 123, 126, 127, 128, 130, 136, and 138 are rejected under 35 U.S.C. 102(a) as being anticipated by de la Fuente et al. (Science, Vol. 276, 6 June 1997, pages 1566-1568.

Claim 1 is drawn to a method for obtaining transgenic plants having an increased capacity to synthesize, to accumulate, and to exude organic acids, by integration into

their genome of a recombinant heterologous DNA molecule encoding enzymes that synthesize organic acids, involving the following steps: a) preparation of a recombinant heterologous DNA molecule encoding one or more genes for enzymes that synthesize organic acids, linked to a promoter sequence functional in plants, and to a transcription termination/polyadenylation sequence functional in plants; b) the transformation of plant cells with the recombinant DNA molecule, and c) the regeneration of transgenic plants starting from transformed cells, or of seeds from plants obtained from these transformed cells, for one or several generations, wherein the genetic information of these transformed cells, or of seeds from plants obtained from these transformed cells, includes the recombinant DNA molecule coding for enzymes that synthesize organic acids. Claims 2 and 75 are drawn to the recombinant DNA molecule comprising one or more microbial genes coding for enzymes that synthesize organic acids. Claims 5 and 78 are drawn to the recombinant molecule comprising one or more bacterial genes. Claims 6 and 79 are drawn to the recombinant molecule comprising a gene that codes for Citrate Synthase. Claims 8 and 82 are drawn to the organic acid being located in the cytoplasm. Claims 11 and 80 are drawn to the molecule comprising a gene of *Pseudomonas aeruginosa* that codes for Citrate Synthase. Claims 12, 13, 16, 17, 19 and 90 are drawn to the transcription termination sequence being Nopaline Synthetase (NOS) gene. Claims 22, 23, 26, 27, 29 and 84 are drawn to the promoter being a constitutive promoter. Claims 62-63, 66-67, 69 and 88 are drawn to the promoter being the 35S promoter of the cauliflower mosaic virus. Claim 74 is drawn to a recombinant, heterologous DNA molecule comprising one or more genes that code for enzymes that

synthesize organic acids, functionally linked to a promoter sequence functional in plants, and to transcription termination/polyadenylation sequence functional in plants.

Claim 92 is drawn to the vector comprising the recombinant DNA molecule of claim 74.

Claims 93, 94, 97, 99, and 101 are drawn to a transgenic plant with increased capacity to synthesize, to accumulate, and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule. Claim 103 is drawn to transgenic plants according to claim 93, tolerant to toxic concentrations of Aluminum.

Claim 104 is drawn to transgenic plants having increased capacity to solubilize or accumulate phosphate. Claim 105 is drawn to transgenic plants having increased capacity to solubilize or accumulate iron. Claim 106 is drawn to transgenic plants requiring less fertilizer for growth. Claim 107 is drawn to the plant developing better or higher productivity in acid soils. Claim 108 is drawn to the plant being a monocot. Claim 109 is drawn to the plant being a dicot. Claims 114-115 are drawn to the plant being *Nicotiana tabacum*. Claims 116-117 are drawn to the plant being *Carica papaya*.

Claims 122, 123, 126-128, 130, 136 and 138 are drawn to a transformed cell or protoplast transformed with the recombinant molecule.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second

column.) Therefore, all of the limitations of the above-mentioned claims are anticipated by de la Fuente et al.

***Claim Rejections - 35 USC § 103***

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 3, 14, 24, 64, 76, 95, and 124 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of John (USPN 6,096,950).

The claims recite the limitation that the DNA molecule comprises a gene of plant origin coding for an enzyme that synthesizes organic acids.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. teaches that the DNA molecule is from a microorganism and not a plant.

John et al. teaches the use of plant genes in transgenic plants. (Col. 2, bridging Col. 3).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from a plant. This is because the gene from a plant would have been readily available and would have been easily assimilated into the transgenic plant.

13. Claims 4, 15, 25, 65, 77, 96, and 125 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Silverman (USPN 5,866,787).

The claims recite the limitation that the DNA molecule comprises a gene of animal origin coding for an enzyme that synthesizes organic acids.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. teaches that the DNA molecule is from a microorganism and not an animal.

Silverman et al. teaches the use of animal genes in transgenic plants. (Col. 7, lines 37-53).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from an

Art Unit: 1655

animal. This is because a gene from an animal would have been readily available and would have been easily assimilated into the transgenic plant.

14. Claims 7, 18, 28, 68, 81, 100, 118-121, 129, and 132 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Lehninger et al. (Biochemistry, Second Edition, 1976, pages 446-447).

The claims are drawn to the recombinant molecule comprising a gene that encodes for malate dehydrogenase.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. do not teach that the organic acid enzyme is malate dehydrogenase.

Lehninger et al. teaches the tricarboxylic acid (TCA) cycle, in which both malic acid is produced from malate dehydrogenase and citric acid is produced from citrate synthase (page 446).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use malate dehydrogenase in the place of citrate synthase. This is because they were both enzymes which produced organic acids which were part of the TCA

cycle, and it would have been obvious that they would have both produced organic acids in a transgenic plant.

15. Claims 9, 10, 20, 21, 30, 31, 70, 71, 83, 102, and 131 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. and further in view of Chou (USPN 6,121,511).

The claims are drawn to the enzyme that synthesizes organic acids being located in the mitochondria or chloroplast.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. do not teach that the enzyme that synthesizes organic acids is located in the mitochondria or chloroplast.

Chou et al. teaches "a fertile transgenic plant is a plant containing a foreign gene stably transformed into its genome including the nuclear, mitochondrial, and/or chloroplast genomes which is capable of transmitting the foreign gene to progeny via sexual reproduction." (Col. 4, liens 54-59).

It would have been obvious to combine de la Fuente and Chou. This is because Chou taught that a transgenic plant was capable of having a foreign gene in the

mitochondria, chloroplast, or cytoplasm. It would have been obvious that, if it were desirable that the organic acid be produced in one of the organelles, to insert the gene into one of those organelles.

16. Claims 32, 33, 36, 37, 39, 85, and 133 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Croy.

The claims are drawn to a root-specific primer.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach a root-specific promoter.

Croy et al. teaches various promoters capable of use with plants, including root-specific promoters. (Abstract).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a root-specific promoter. This is because it would have been desirable that the organic acid be exuded from the roots, and therefore it would have been obvious to use a root-specific promoter in order to produce organic acid from the roots of the plant.

Art Unit: 1655

17. Claims 42, 43, 46, 47, 49, 86, and 134 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Mucchal (Proc. Natl Acad. Sci, Vol. 93, pages 10519-10523, 1996).

The claims are drawn to the promoter being inducible by stress caused by low phosphate availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach that the promoter is inducible by stress caused by low phosphate availability.

Mucchal et al. teaches "an increase in phosphate uptake rate of roots and cultured and cultured cells has been observed in several plant species....Phosphate stress in yeast results in activation/inactivation of several genes associated with the pho-regulation, leading to enhanced synthesis of the high-affinity phosphate transporter and phosphatases." (page 10519, first col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Mucchal, so that the promoter is induced by stress caused by low phosphate availability. This is because

phosphate is one of the major nutrients required by plants and it would have been obvious to increase the rate of phosphate uptake when the organic acid was being released in order to minimize stress on the plant.

18. Claims 52, 53, 56, 57, 59, 105, 135, and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Guerinot et al. (USPN 5,846,821).

The claims are drawn to the promoter being inducible by stress caused by low iron availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al does not teach that the promoter is a promoter inducible by stress caused by low iron availability.

Guerinot et al. teaches "many iron-efficient plant varieties have iron uptake strategies...that, not surprisingly, are directed at solubilizing iron....most iron deficient plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases." (Col. 1, lines 25-35).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Guerinot, so that the promoter is induced by stress caused by low iron availability. This is because iron is one of the major nutrients required by plants and it would have been obvious to increase the rate of iron uptake when the organic acid was being released in order to minimize stress on the plant.

19. Claims 110-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Foulkes et al. (USPN 6,136,779).

The claims are drawn to the transgenic plants belonging to any one of the families Poaceae, Lileaceae, Leguminoseae, Solanaceae, Caricaceae, Cucurbitaceae, Triticum, Oryza, Zea, Sorghum, Avena, Saccharum, Solanum, Lycoperisicum, or Glycine.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente does not teach that the transgenic plant is from one of the above-mentioned families.

Foulkes et al. teaches a large variety of plant and animal species which are transgenically altered to express the citric acid gene. (Col. 30, lines 35-60).

It would have been obvious to one of ordinary skill in the art that a variety of plant species were modifiable by a gene encoding an enzyme that encodes an organic acid. This is because many plants were known to be susceptible to increased levels of aluminum, or decreased levels of phosphate and iron, and it would have been obvious that exuding organic acid would have been beneficial to their growth and development.

20. Claim 54 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente in view of John, as disclosed above, and further in view of Guerinot.

The claim recites the limitation that the DNA molecule comprises a gene of plant origin coding for an enzyme that synthesizes organic acids, and that the promoter is inducible by stress caused by low iron availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.) De la Fuente et al. teaches that the DNA molecule is from a microorganism and not a plant.

De la Fuente does not teach that the promoter is inducible by stress caused by low iron availability.

John et al. teaches the use of plant genes in transgenic plants. (Col. 2, bridging Col. 3).

John et al. does not teach that the promoter is inducible by stress caused by low iron availability.

Guerinot et al. teaches "many iron-efficient plant varieties have iron uptake strategies...that, not surprisingly, are directed at solubilizing iron....most iron deficient plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases." (Col. 1, lines 25-35).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from a plant. This is because the gene from a plant would have been readily available and would have been easily assimilated into the transgenic plant. Furthermore, It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Guerinot, so that the promoter is induced by stress caused by low iron availability. This is because iron is one of the major nutrients required by plants and it would have been obvious to increase the rate of iron uptake when the organic acid was being released in order to minimize stress on the plant.

21. Claim 55 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente in view of Silverman, as disclosed above, and further in view of Guerinot.

The claim recites the limitation that the DNA molecule comprises a gene of animal origin coding for an enzyme that synthesizes organic acids, and that the promoter is inducible by stress caused by low iron availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer AI tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach that the DNA molecule is from an animal. De la Fuente does not teach that the promoter is inducible by stress caused by low iron availability.

Silverman et al. teaches the use of animal genes in transgenic plants. (Col. 7, lines 37-53).

Silverman et al. does not teach that the promoter is inducible by stress caused by low iron availability.

Guerinot et al. teaches "many iron-efficient plant varieties have iron uptake strategies...that, not surprisingly, are directed at solubilizing iron....most iron deficient plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases." (Col. 1, lines 25-35).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from animal. This is because the gene from an animal would have been readily available and would have been easily assimilated into the transgenic plant. Furthermore, It would

have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Guerinot, so that the promoter is induced by stress caused by low iron availability. This is because iron is one of the major nutrients required by plants and it would have been obvious to increase the rate of iron uptake when the organic acid was being released in order to minimize stress on the plant.

22. Claim 58 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente in view of Lehninger, as disclosed above, and further in view of Guerinot et al.

The claim recites the limitation that molecule comprises a gene that codes for the enzyme malate dehydrogenase, and that the promoter is inducible by low iron availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach that the DNA molecule codes for malate dehydrogenase. De la Fuente does not teach that the promoter is inducible by stress caused by low iron availability.

Lehninger et al. teaches the tricarboxylic acid (TCA) cycle, in which both malic acid is produced from malate dehydrogenase and citric acid is produced from citrate synthase (page 446).

Lehninger et al. does not teach that the promoter is inducible by stress caused by low iron availability.

Guerinot et al. teaches "many iron-efficient plant varieties have iron uptake strategies...that, not surprisingly, are directed at solubilizing iron....most iron deficient plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases." (Col. 1, lines 25-35).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use malate dehydrogenase in the place of citrate synthase. This is because they were both enzymes which produced organic acids which were part of the TCA cycle, and it would have been obvious that they would have both produced organic acids in a transgenic plant. Furthermore, It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Guerinot, so that the promoter is induced by stress caused by low iron availability. This is because iron is one of the major nutrients required by plants and it would have been obvious to increase the rate of iron uptake when the organic acid was being released in order to minimize stress on the plant.

23. . Claims 60 and 61 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente in view of Chou, as disclosed above, and further in view of Guerinot et al.

The claims are drawn to the enzyme that synthesizes organic acids being located in the mitochondria or chloroplast. The claims also recite that the promoter is inducible by low iron availability.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. do not teach that the enzyme that synthesizes organic acids is located in the mitochondria or chloroplast. De la Fuente does not teach that the promoter is inducible by stress caused by low iron availability.

Chou et al. teaches "a fertile transgenic plant is a plant containing a foreign gene stably transformed into its genome including the nuclear, mitochondrial, and/or chloroplast genomes which is capable of transmitting the foreign gene to progeny via sexual reproduction." (Col. 4, liens 54-59).

Chou et al. does not teach that the promoter is inducible by stress caused by low iron availability.

Guerinot et al. teaches "many iron-efficient plant varieties have iron uptake strategies...that, not surprisingly, are directed at solubilizing iron....most iron deficient

plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases." (Col. 1, lines 25-35).

It would have been obvious to combine de la Fuente and Chou. This is because Chou taught that a transgenic plant was capable of having a foreign gene in the mitochondria, chloroplast, or cytoplasm. It would have been obvious that, if it were desirable that the organic acid be produced in one of the organelles, to insert the gene into one of those organelles. Furthermore, It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Guerinot, so that the promoter is induced by stress caused by low iron availability. This is because iron is one of the major nutrients required by plants and it would have been obvious to increase the rate of iron uptake when the organic acid was being released in order to minimize stress on the plant.

24. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of John, and further in view of Croy.

The claims are drawn to a root-specific primer, and to the gene being of plant origin.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower

mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach a root-specific promoter or a gene of plant origin.

Croy et al. teaches various promoters capable of use with plants, including root-specific promoters. (Abstract).

Croy et al. does not teach the use of plant genes in transgenic plants.

John et al. teaches the use of plant genes in transgenic plants. (Col. 2, bridging Col. 3).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from a plant. This is because the gene from a plant would have been readily available and would have been easily assimilated into the transgenic plant. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a root-specific promoter. This is because it would have been desirable that the organic acid be exuded from the roots, and therefore it would have been obvious to use a root-specific promoter in order to produce organic acid from the roots of the plant.

25. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Silverman, and further in view of Croy.

The claims are drawn to a root-specific primer, and to the gene being of animal origin.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach a root-specific promoter or a gene of animal origin.

Croy et al. teaches various promoters capable of use with plants, including root-specific promoters. (Abstract).

Croy et al. does not teach a gene of animal origin.

Silverman et al. teaches the use of animal genes in transgenic plants.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from an animal. This is because the gene from an animal would have been readily available and would have been easily assimilated into the transgenic plant. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a root-specific promoter. This is because it would have been desirable that the organic acid was exuded from the roots, and therefore it would have been obvious to use a root-specific promoter in order to produce organic acid from the roots of the plant.

26. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Lehringer, and further in view of Croy.

The claims are drawn to a root-specific primer, and to the enzyme being malate dehydrogenase.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.) Therefore, all of the limitations of the above-mentioned claims are anticipated by de la Fuente et al.

De la Fuente et al. does not teach a root-specific promoter or a gene of plant origin. De la Fuente et al. do not teach that the organic acid enzyme is malate dehydrogenase.

Lehninger et al. teaches the tricarboxylic acid (TCA) cycle, in which both malic acid is produced from malate dehydrogenase and citric acid is produced from citrate synthase (page 446).

Lehringer et al. does not teach root-specific promoters.

Croy et al. teaches various promoters capable of use with plants, including root-specific promoters. (Abstract).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use malate dehydrogenase in the place of citrate synthase. This is because they were both enzymes which produced organic acids which were part of the TCA cycle, and it would have been obvious that they would have both produced organic acids in a transgenic plant. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a root-specific promoter. This is because it would have been desirable that the organic acid was exuded from the roots, and therefore it would have been obvious to use a root-specific promoter in order to produce organic acid from the roots of the plant.

27. Claims 40 and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Chou, and further in view of Croy.

The claims are drawn to a root-specific primer, and to the enzyme that synthesizes organic acids being in the chloroplasts or mitochondria.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach a root-specific promoter or that the enzyme that synthesizes organic acids is located in the mitochondria or chloroplast.

Chou et al. teaches "a fertile transgenic plant is a plant containing a foreign gene stably transformed into its genome including the nuclear, mitochondrial, and/or chloroplast genomes which is capable of transmitting the foreign gene to progeny via sexual reproduction." (Col. 4, liens 54-59).

Chou et al. does not teach root-specific promoters.

Croy et al. teaches various promoters capable of use with plants, including root-specific promoters. (Abstract).

It would have been obvious to combine de la Fuente and Chou. This is because Chou taught that a transgenic plant was capable of having a foreign gene in the mitochondria, chloroplast, or cytoplasm. It would have been obvious that, if it were desirable that the organic acid be produced in one of the organelles, to insert the gene into one of those organelles. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a root-specific promoter. This is because it would have been desirable that the organic acid was exuded from the roots, and therefore it would have been obvious to use a root-specific promoter in order to produce organic acid from the roots of the plant.

28. Claim 44 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of John, and further in view of Muchhal.

The claims are drawn to a promoter inducible by low phosphate availability, and to the gene being of plant origin.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco

and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach low phosphate availability or a gene of plant origin.

John et al. teaches the use of plant genes in transgenic plants. (Col. 2, bridging Col. 3).

John et al. does not teach low phosphate availability triggered promoters.

Mucchal et al. teaches "an increase in phosphate uptake rate of roots and cultured and cultured cells has been observed in several plant species....Phosphate stress in yeast results in activation/inactivation of several genes associated with the pho-regulation, leading to enhanced synthesis of the high-affinity phosphate transporter and phosphatases." (page 10519, first col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Mucchal, so that the promoter is induced by stress caused by low phosphate availability. This is because phosphate is one of the major nutrients required by plants and it would have been obvious to increase the rate of phosphate uptake when the organic acid was being released in order to minimize stress on the plant. It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme

that synthesizes organic acid from a plant. This is because the gene from a plant would have been readily available and would have been easily assimilated into the transgenic plant.

29. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Silverman, and further in view of Mucchal.

The claims are drawn to a promoter inducible by low phosphate availability, and to the gene being of animal origin.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach a low phosphate inducible promoter, or a gene of animal origin.

Silverman et al. teaches the use of animal genes in transgenic plants.

Silverman does not teach a low phosphate inducible promoter.

Mucchal et al. teaches "an increase in phosphate uptake rate of roots and cultured and cultured cells has been observed in several plant species....Phosphate stress in yeast results in activation/inactivation of several genes associated with the

pho-regulation, leading to enhanced synthesis of the high-affinity phosphate transporter and phosphatases." (page 10519, first col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Mucchal, so that the promoter is induced by stress caused by low phosphate availability. This is because phosphate is one of the major nutrients required by plants and it would have been obvious to increase the rate of phosphate uptake when the organic acid was being released in order to minimize stress on the plant. It would have been obvious to one of ordinary skill in the art at the time of the invention to use a gene encoding the enzyme that synthesizes organic acid from an animal. This is because the gene from an animal would have been readily available and would have been easily assimilated into the transgenic plant.

30. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Lehringer, and further in view of Mucchal.

The claims are drawn to a promoter inducible by stress caused by low phosphate levels, and to the enzyme being malate dehydrogenase.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower

mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach low phosphate induced promoter, or a gene of plant origin.

Lehninger et al. teaches the tricarboxylic acid (TCA) cycle, in which both malic acid is produced from malate dehydrogenase and citric acid is produced from citrate synthase (page 446).

Lehringer et al. does not teach phosphate-induced promoters.

Mucchal et al. teaches "an increase in phosphate uptake rate of roots and cultured and cultured cells has been observed in several plant species....Phosphate stress in yeast results in activation/inactivation of several genes associated with the pho-regulation, leading to enhanced synthesis of the high-affinity phosphate transporter and phosphatases." (page 10519, first col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Mucchal, so that the promoter is induced by stress caused by low phosphate availability. This is because phosphate is one of the major nutrients required by plants and it would have been obvious to increase the rate of phosphate uptake when the organic acid was being released in order to minimize stress on the plant. It would have been obvious to one of ordinary skill in the art at the time of the invention to use malate dehydrogenase in the place of citrate synthase. This is because they were both enzymes which produced

organic acids which were part of the TCA cycle, and it would have been obvious that they would have both produced organic acids in a transgenic plant.

31. Claims 50 and 51 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Chou, and further in view of Mucchal.

The claims are drawn to a stress-inducible promoter caused by low phosphate availability, and to the enzyme that synthesizes organic acids being in the chloroplasts or mitochondria.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach phosphate-inducible promoters or that the enzyme that synthesizes organic acids is located in the mitochondria or chloroplast.

Chou et al .teaches "a fertile transgenic plant is a plant containing a foreign gene stably transformed into its genome including the nuclear, mitochondrial, and/or chloroplast genomes which is capable of transmitting the foreign gene to progeny via sexual reproduction." (Col. 4, liens 54-59).

Chou et al. does not teach low phosphate inducible promoters.

Mucchal et al. teaches "an increase in phosphate uptake rate of roots and cultured and cultured cells has been observed in several plant species....Phosphate stress in yeast results in activation/inactivation of several genes associated with the pho-regulation, leading to enhanced synthesis of the high-affinity phosphate transporter and phosphatases." (page 10519, first col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of de la Fuente with those of Mucchal, so that the promoter is induced by stress caused by low phosphate availability. This is because phosphate is one of the major nutrients required by plants and it would have been obvious to increase the rate of phosphate uptake when the organic acid was being released in order to minimize stress on the plant. It would have been obvious to combine de la Fuente and Chou. This is because Chou taught that a transgenic plant was capable of having a foreign gene in the mitochondria, chloroplast, or cytoplasm. It would have been obvious that, if it were desirable that the organic acid be produced in one of the organelles, to insert the gene into one of those organelles.

32. Claims 72, 73, 89, and 137 are rejected under 35 U.S.C. 103(a) as being unpatentable over de la Fuente et al. in view of Chou, and further in view of Lundquist et al. (USPN 5,990,390).

The claims are drawn to the recombinant molecule comprising a signal peptide sequence to direct a heterologous enzyme that synthesizes organic acids to the chrloroplast or the mitochondria of the transgenic cell.

De la Fuente et al. teaches "to examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that over-express a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm... We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene fused to the 35S promoter from the cauliflower mosaic virus and nos [Nopaline Synthetase] 3' end sequences." (Page 1566, second column.)

De la Fuente et al. does not teach transit peptides or that the enzyme that synthesizes organic acids is located in the mitochondria or chloroplast.

Chou et al. teaches "a fertile transgenic plant is a plant containing a foreign gene stably transformed into its genome including the nuclear, mitochondrial, and/or chloroplast genomes which is capable of transmitting the foreign gene to progeny via sexual reproduction." (Col. 4, liens 54-59).

Chou et al. does not teach transit peptides.

Lundquist et al. teaches "additionally, vectors may be constructed and employed in the intracellular targeting of a specific gene product within the cells of a transgenic plant or in directing a protein to the extracellular environment. This will generally be achieved by joining a DNA sequence encoding a transit or signal peptide sequence to the coding sequence of a particular gene. The resultant transit, or signal, peptide will transport the protein to a particular intracellular, or extracellular destination, respectively, and will then be post-translationally removed. Transit or signal peptides act by facilitating the transport of proteins through intracellular membranes, e.g., vacuole,

vesicle, plastid, and mitochondrial membranes, whereas signal peptides direct protein through the extracellular membrane" (Col. 12, lines 44-57).

It would have been obvious to combine de la Fuente and Chou. This is because Chou taught that a transgenic plant was capable of having a foreign gene in the mitochondria, chloroplast, or cytoplasm. It would have been obvious that, if it were desirable that the organic acid be produced in one of the organelles, to insert the gene into one of those organelles. Furthermore, it would have been obvious to use a signal peptide to transport the gene into the desired organelle. This is because it would have been necessary to transport the gene into the correct organelle, if it was desired that it be expressed there, and it would have also been necessary to use a signal peptide to transport the gene.

### *Summary*

Claims 1-73, 81, and 129 and 132 are objected to. Claims 92-107 are rejected under 35 U.S.C. 112, second paragraph. Claims 1, 2, 5, 6, 8, 11-13, 16, 17, 19, 22, 23, 26, 27, 29, 62, 63, 66, 67, 69, 74, 75, 78, 79, 80, 82, 84, 88, 90-94, 97-99, 101, 103, 104, 107-109, 114-117, 122, 123, 126, 127, 128, 130, 136, and 138 are rejected under 35 U.S.C. 102(a) Claims 3, 4, 7, 9, 10, 14-15, 18, 20, 21, 24, 25, 28, 30-61, 68, 69-73, 76, 77, 81, 83, 85-87, 89, 95, 96, 100, 102, 105, 110-113, 118-121, 124, 125, 129, 131-135, and 137 are rejected under 35 U.S.C. 103(a). No claims are free of the prior art.

### *Conclusion*

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janell Taylor Cleveland, whose telephone number is (703) 305-0273.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached at (703) 308-1152.

Any inquiries of a general nature relating to this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed to Group 1634 via the PTO Fax Center using (703) 305-3014 or 305-4227. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (November 15, 1989.)

Janell Taylor Cleveland

June 8, 2001

*Carla Myers*  
CARLA J. MYERS  
PRIMARY EXAMINER